UNIT VI - PLANT TECHNOLOGIES

Lesson 2: Plant Tissue Culture

Competency/Objective: Explain the process of tissue culture.

Study Questions

1. **What is plant tissue culture?**
2. **What are the advantages and disadvantages of plant tissue culture?**
3. **What equipment is needed for plant tissue culture?**
4. **What steps are involved in plant tissue culture?**
5. **What are the four stages of tissue culture growth?**
6. **How is plant tissue culturing used in genetic engineering?**

References

1. *Biotechnology: Applications in Agriculture (Student Reference)*. University of Missouri-Columbia: Instructional Materials Laboratory, 1998, Unit VI.
2. Activity Sheets
   a) AS 2.1: Tissue Culturing Strawberries (Instructor)
   b) AS 2.1: Tissue Culturing Strawberries (Student)

Lesson 2: Plant Tissue Culture

TEACHING PROCEDURES

A. Review

Traditional plant breeding techniques, such as selective breeding, have increased plant quality and production. However, a method of propagation called tissue culture is providing plant breeders with new tools for crop improvement. Tissue culture has not replaced traditional breeding practices but rather has enhanced their effectiveness.

B. Motivation

Plant tissue culture was first used in the early 1970s in the propagation of orchids. The orchid seed is very difficult to sow and germinate. It is not only the smallest seed in the world, but it must be exposed to a specific fungus before it will germinate. To produce an orchid from a seed takes an average of seven years. Tissue culture became the solution to the difficulties of orchid propagation. Today, tissue culture is a commonly used and vitally important method of plant reproduction.

Purchase a miniature plant grown from tissue culture. Use this tiny plant to introduce the basic concept of tissue culture. Every plant cell has the entire genetic code and is capable of producing an entire plant.

C. Assignment

D. Supervised Study

E. Discussions

1. Ask students to define the word tissue. Explain that a tissue is a group of cells that function together for a purpose. Examples of plant tissue include the leaf, stem, root, bud, etc.

   What is plant tissue culture?

   Plant tissue culture can be defined as an asexual method of reproduction in which a piece of a parent plant is placed in a sterile artificial media where it grows into a new plant.

2. Ask students why tissue culture is done. Have students speculate about the advantages and disadvantages of using tissue culture.

   What are the advantages and disadvantages of plant tissue culture?

   a) Advantages
      1) Allows for mass propagation of clones of a desirable plant
      2) Allows the production of pathogen-free plants
      3) Conserves time through the year-round propagation of plants

4) Conserves growing space

b) Disadvantages
   1) Requires expensive, sophisticated equipment and facilities
   2) Susceptibility to contamination by microorganisms
   3) Requires skilled workers, which adds to the total cost

3. Show students a petri dish and an autoclave or pressure cooker. Ask students to describe how an autoclave works. Explain that an autoclave uses steam under pressure (approximately 15 psi) to heat items above the temperature that microorganisms can survive.

What equipment is needed for plant tissue culture?

a) Preparation
   1) Refrigerator
   2) pH meter
   3) Scale or balance
   4) Heating plate
   5) Autoclave

b) Transfer
   1) Fume or air flow hood
   2) Forceps
   3) Scalpel
   4) Test tubes or petri dishes
   5) Dissecting microscope

c) Growth
   1) Growth chamber - a room that controls exposure to heat and light

4. Ask students to list the steps of the procedure for tissue culturing.

What steps are involved in plant tissue culture?

a) Media preparation
   1) The growing media for tissue culture varies in composition with the species of plant being used.
   2) Generally the media contains plant nutrients, mineral salts with vitamins, hormones, pure water, sugar, and agar (if a semi-solid media is needed)

b) Selecting and collecting an explant to be cultured
   1) Several types of plant tissues are used for explants.
      (a) Shoot tip
      (b) Bud
      (c) Leaf with veins
      (d) Node
      (e) Bud scale
   2) Rapidly growing tissues are usually preferred for the explant.
   3) Tissue must be healthy and disease free.

c) Cleaning the explant
   1) Plant tissue is disinfected
      (a) Alcohol is used on woody plants only.

(b) For other plants, the explant is soaked in a 10 percent bleach solution for 10 minutes; plant tissue will be damaged if it is soaked too long.
(c) A drop or two of detergent is often added to the bleach solution as a wetting agent.

2) After the tissue is disinfected, it is rinsed in pure water at least three times.

d) Transferring explants to growing media
1) The explant sections are trimmed and transferred to the growing media; some tissue types are divided for growing more plants.
2) A dissecting microscope is sometimes needed for very small explant tissues.
3) This procedure must take place in a sterile environment.

5. Ask students to explain each of the four stages of tissue culture growth.

What are the four stages of tissue culture growth?

a) Initiation and establishment (four to six weeks)
1) A callus, made of rapidly dividing cells, forms and grows in response to the wounding or cutting of the plant tissue.
2) Shoots or immature stems begin to grow.

b) Proliferation or multiplication (one to three months)
1) The shoot multiplies into many shoots.
2) The new shoots can be divided to increase the number of plants produced.
3) This stage uses a slightly different growing media.

c) Pretransplant (three weeks)
1) Roots begin to grow.
2) A slightly different media is used.
3) More light is required.
4) Young plants are stressed slightly in a process called hardening off, in which young plants are exposed to conditions outside the sterile container.

d) Transplanting
1) The growing plants are put in pots and moved to a shady, humid greenhouse.
2) The plants are hardened off again and then moved to a regular greenhouse to receive full sun and less humidity.

6. Have students identify reasons that a person or company working with developing transgenic plants would want to use tissue culture.

How is plant tissue culturing used in genetic engineering?

a) Genetically modified plant tissues can be rapidly grown into plants.
b) A large number of plants can be screened for the presence of a desirable trait.

F. Other Activities

1. Demonstrate media preparation.

2. Tissue culture kits and African violet kits are available for purchase from Carolina Biological or Fisher Scientific. Follow the directions on the kits when preparing them.

3. Show the videos *Plant Tissue Culture Part 1 & 2* (VEP) and *Introduction to Plant Tissue Culture* (CEV), all of which are available from MVRC.

G. Conclusion

More than 50 plant species have been genetically modified. In almost every case, tissue culture played an important role in recovering the modified plant tissues. In 1990, more than 300 commercial plant tissue culture labs were in operation. As plant breeders continue to make use of tissue culture techniques, the use of tissue culture will likely increase.

H. Answers to Activity Sheet

I. Answers to the Evaluation

1. b
2. d
3. c
4. b
5. c
6. c
7. d

8. Plant tissue culture can be defined as an asexual method of reproduction in which a piece of a parent plant is placed in a sterile artificial media where it grows into a new plant.

9. Tissue culturing is used in genetic engineering to rapidly grow genetically modified plant tissues into plants and to screen a large number of plant tissues for the presence of a desirable trait.

10. Students may list any two of the following: refrigerator, pH meter, scale or balance, heating plate, autoclave, fume or air flow hood, forceps, scalpel, petri dishes or test tubes, dissecting microscope, or growth chamber.

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EVALUATION

Circle the letter that corresponds to the best answer.

1. Which of the following statements is not an advantage of tissue culture?

   a. Tissue culture allows for the mass propagation of clones.
   b. Plant tissue cultures are susceptible to contamination.
   c. Plant tissue culture allows for the production of pathogen-free plants.
   d. Plant tissue culture allows for the propagation of plants year round.

2. Which of the following substances is not commonly used to make a tissue culture media?

   a. Sugar
   b. Mineral salts with vitamins
   c. Plant hormones
   d. A dilute sodium chloride solution

3. Which of the following is an important criterion to consider when selecting an explant?

   a. The explant should be selected from a young parent plant.
   b. The parent plant should be a monocot because dicot plants do not give good explants.
   c. The explant should be taken from a healthy, rapidly growing part of the parent plant.
   d. The explant is always taken from the shoot tip of the parent plant.

4. If it is not woody plant tissue, the selected explant is disinfected with:

   a. Alcohol.
   b. 10 percent bleach solution.
   c. A detergent.
   d. A very weak acid solution.

5. In which stage of plant tissue growth do the roots begin to form and grow?

   a. Initiation and establishment
   b. Proliferation or multiplication
   c. Pretransplant
   d. Transplanting

6. The stage in which the shoot begins to grow is called the:

   a. Transplanting stage.
   b. Proliferation or multiplication stage.
   c. Initiation and establishment stage.
   d. Pretransplanting stage.

7. A callus is a:

   a. Mass of dead plant cells that accumulate as the tissue culture grows.
   b. Group of cancer cells that can destroy a plant tissue culture.
   c. Group of old cells that forms when the incorrect part of the plant is selected for the explant.
   d. Group of rapidly dividing cells that is a response to the wounding of plant tissue.

Complete the following short answer questions.

8. What is plant tissue culture?

9. How is plant tissue culture used in genetic engineering?

10. What are two pieces of equipment used in tissue culture?

Lesson 2: Plant Tissue Culture Name _____

Tissue Culturing Strawberries

Objective: Perform plant tissue culture.

Materials and Equipment:

1 medium-sized stainless steel pan
1 long-handled spoon
1-2 glass test tubes with caps (25 x 100 mm)
Test tube racks
2 gallons of sterile distilled bottled water
1 glass (Pyrex) quart pitcher or old coffee pot
1 pressure cooker
2-4 eight-inch forceps
1 sharp knife or scalpel
Parafilm™ or clear tape
Rubber or latex gloves
20 ml of 1N NaOH
20 ml of 1N HCl
2 eyedroppers
1 plastic graduated cylinder
1 small bottle of household bleach
2 packages of premixed powder tissue culture medium (shoot multiplication media and pretransplant media)
1 package of agar
1 package of litmus paper (3.5-6.8) or a pH meter
2-4 medium-sized plastic containers
2-3 sterile paper towels (made by rolling them up in aluminum foil and sterilizing them in the pressure cooker)
1 72-hole seedling tray (11 x 22)
Shelves lighted by cool white fluorescent lights
1 transfer chamber (It can be made from wood or cardboard with a clear plastic sheet or plexiglass over the front and top of the chamber. Holes should be cut in the front so that both hands can be used to work inside the chamber.)
10-20 strawberry runner tips (about 1 inch long)

Procedure for Media Preparation:

1. Follow the directions on the prepackaged shoot multiplication media mix to prepare the media. About one liter of media is enough for the entire class. Make sure that a 2-liter container or larger is used when mixing the media since it can boil up and spill. Add the powder mix to the water (not the reverse). Stir the mixture.

2. Next, the pH of the mixture must be adjusted to 5.7. Measure the pH of the solution with litmus paper. If the solution has a pH higher than 5.7, add one drop of HCl and stir the

solution. If, however, the solution has a pH lower than 5.7, add one drop of NaOH and stir. This process is repeated until the pH is 5.7.

3. Thicken the mix by adding agar. Approximately 5 grams of agar are needed per liter of solution. After the agar is stirred into the solution, heat it and stir until the solution becomes clear. Transfer the hot solution to the glass pitcher and then carefully pour it into the test tubes.

4. Sterilize the medium in a pressure cooker for about 15 minutes. When sterilizing test tubes holding media, place them in a wide-mouth jar or tie them in bundles of ten so that they stand up in the pressure cooker.

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10-20 strawberry runner tips (about 1 inch long)

Procedure:

A. Selecting and Cleaning the Explants

1. Select a healthy-looking strawberry runner tip on which the bud has not yet opened. Cut off 1 to 1 1/2 inches of the tip and place it in a plastic bag containing a damp paper towel.

2. Fill a wide-mouth jar with 1/2 pint of sterile water. Add 2 or 3 drops of liquid dishwashing detergent. Place the runner tips in the jar, put the lid on, and vigorously

shake the jar for 1 minute. Pour out the water and rinse the runner tips 2 or 3 times with sterile water. Repeat this process, or dip the runner tips in 70 percent alcohol for only a few seconds and then rinse 2 to 3 times with sterile water.

3. In another container, add 30 ml of bleach to 270 ml of sterile water to yield a 10 percent bleach solution. Add 2 drops of the detergent and place the explant in the solution. Shake for 10 seconds every minute for ten minutes. Quickly drain the solution; add sterile water and shake the container.

B. Transferring the Explants

1. Spray and wipe down all the inside surfaces of the transfer chamber with a 10 percent bleach solution. Allow them to air dry. Place a small container of 10 percent bleach solution and another container of 1 percent bleach solution in the transfer chamber to use for sterilizing the instruments and gloved hands. Place the forceps and knife in the 10 percent bleach solution and then in the 1 percent solution. Lay the sterilized instruments on a sterile paper towel and allow them to air dry.

2. Using the forceps, unroll another paper towel on the work area inside the transfer chamber. Use the forceps to place the runner tip on the towel. Hold the tip with the forceps while picking up the knife or scalpel and cutting off 1 cm of the stem. Put the knife in the 10 percent bleach solution. Pick up a test tube of medium while holding the explant with the forceps. Remove the test tube cap with the small finger of one hand and hold it firmly in place while putting the cut explant on the medium. Cap the test tube and seal it with Parafilm™ or tape.

C. Growing the Cultures

1. Place the test tube in the planter tray or another holder on a shelf under a florescent light that is 8 to 10 inches away. Continuous light may be used, but if a timer is available, 16 hours of light is normal. Plants may remain at room temperature.

2. Check the cultures every day for signs of contamination. If any fungus or other form of contamination is present, sterilize the contaminated test tubes before discarding.

3. Transfer the explant to new medium every two weeks until it is growing well.

4. In one to two months, the explants should have many shoots and can be divided. When shoots are observed, divide the explant into two pieces about 0.5 cm in diameter. Repeat this process until enough growing plants are produced. Transfer them to a pretransplant medium that has no hormones.

5. After two to four weeks on the medium, the roots should develop. Transplant the plants to an artificial soil mix (greenhouse mix) in a seedling tray. Cover them with transparent plastic and place on a lighted shelf or in a shaded greenhouse.

6. After two or three weeks, uncover the tray daily for a length of time. Gradually increase the time it remains uncovered for a week until they are no longer being covered.